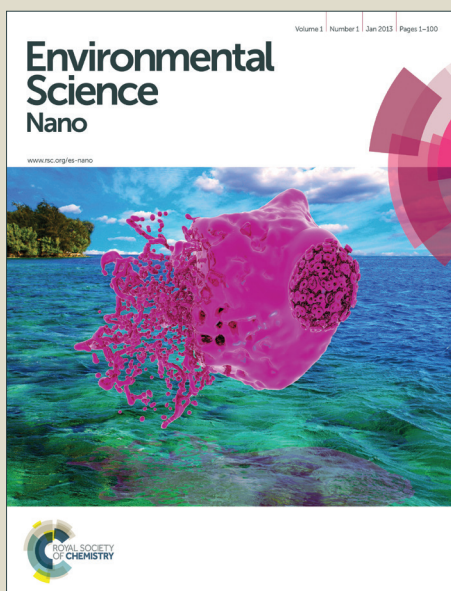


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ARTICLE

Influence of different types of natural organic matter on titania nanoparticles stability: effects of counter ion concentration and pH

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The effects of biopolymeric (alginate) and refractory macromolecules (humic and fulvic acids) on the aggregation kinetics of anatase titanium dioxide (titania) nanoparticles were evaluated. The particles were synthesized using a wet-chemical method based on the hydrolysis of TiCl_4 . Stable suspensions of positively-charged titania were obtained at pH 2.5. One batch of this product was shifted above of the point of zero charge to pH 12. These dispersions were mixed with three different types of well characterized environmental macromolecules, sodium alginate, fulvic acid and humic acid, and evaluated in terms of the stability. Changes in particle size were measured using time-resolved dynamic light scattering (TR-DLS) at different concentrations of three electrolytes: NaCl, CaCl_2 and Na_2SO_4 and different solution pH. Results were in agreement with DLVO calculations. The use of TR-DLS for determining aggregation rates is critically discussed.

Nanoimpact:

The stability of titania nanoparticles in natural waters is influenced by the concentration of nanoparticles themselves and the physico-chemical characteristics of the receiving water. Predominantly, electrolytes and pH determine the extent of repulsion by electrostatic or electro-steric mechanisms and natural organic matter can provide some additional stabilization or cause aggregation depending on the surface coverage of the particles. This work examines the effects of these parameters on the stability of titania nanoparticles for three types of macromolecules and three electrolytes commonly found in natural waters. Implications of this study are important mainly for near source emissions, spill situations and ecotoxicity tests, where other natural or anthropogenic particles are relatively less significant for determining the ENP fate.

Introduction

Nanoparticles and nanomaterials are becoming increasingly common in products for multiple applications¹. Therefore, the environmental fate of these novel materials and their transformation products must be predicted in a physically meaningful way^{2,3}.

Nanoparticles (NP) in aquatic suspensions are subject to different types of molecular forces that are dependent on the material, the hydraulic conditions and the presence of dissolved substances; these forces can lead to aggregation, stabilization or deposition of the particles in the dispersion. The interactions between the forces encompass the particles, the media in which they are dispersed and other surfaces. In many cases of environmental interest the main forces that dominate the

stability of the dispersion are long-ranged Van der Waals forces, short-ranged electrostatic double-layer forces and steric interactions and these are modulated by hydrodynamic interactions⁴. All these forces are ubiquitous in environmental waters and ecotoxicological growth media tests, where macromolecules, ions and surfaces are abundant.

Depending on the entry point into the environment the concentration of particles may be high or low, punctual or dispersed. Transformation processes will act concomitant on the particles with different degree that depends on the physical details of the release. One of these transformation processes involves the sorption of ubiquitous natural organic matter (NOM) present in natural waters, onto the surface of the engineered NP (ENP); this sorption can lead to aggregation via bridging mechanisms or stabilisation through electro-steric or

solely steric effects. The ratio between NOM to ENP, the nature of the specific NOM and ENP surface and the concentrations of both will be determinant of the extent to which any of these processes will be favoured.

Titania (TiO_2) NP are currently used in a great number of consumer products and the likelihood that individual NP or some of the transformation products reaches the environment is increasing^{5, 6}. The effects of titania in the environment are currently being documented. Since NP in the environment will undergo aggregation due to the forces aforementioned, then the exposure to titania NP is going to be related to the extent of aggregation.

In this study the stability of synthesized titania NP obtained at pH 2.5 and pH 12 was evaluated. These dispersions were mixed with three different types of organic environmental substances, Sodium Alginate (ALG), Fulvic acid (FA) and Humic acid (HA), and evaluated in terms of the stability. The changes in particle size were monitored with time-resolved dynamic light scattering (TR-DLS) at different concentrations of three electrolytes: NaCl, CaCl_2 and Na_2SO_4 and different solution pH.

This new set of data will help to elucidate the behaviour of engineered nanoparticles in aquatic systems where the presence of other particulate matter is not crucial, e.g. ecotoxicological tests or spill situations. Additionally, a new methodology for the use of TR-DLS for determining aggregation rates measuring simultaneously size and intensity variations with conventional DLS apparatus is provided.

Previous studies on titania stability and NOM sorption.

Several studies have been carried out concerning aggregation behaviour of titania or other metal oxides. Most of those related to titania NP have been summarized in the review by Liu et al⁷ and some of their findings have been compiled and complemented with factors that were considered important according to the focus of the present paper (See Table S1 in the supplementary information†). Some studies have focused on the effects that NOM has on the final stable suspension while some others have focused on the effects of electrolytes on the stability of NP. Contrastingly, we have investigated the effect of electrolytes with two different valence states on particles stabilized after mixing with NOM at different pH levels.

Sedimentation rates and aggregation kinetics are sometimes difficult to compare between experiments even if the same source of material is used. The dispersion of the particles in the media may have different outcomes in terms of particle size distribution (PSD) and particle number concentration (even if mass concentrations are reported, the number concentration remains unknown); since these two parameters are influential on the collision rates (i.e. more particles will have more encounters and smaller particles will surpass more easily the energy barriers⁸) then the comparison between experiments is difficult to be made in a straightforward way. Furthermore, the use of hydrolytically synthesized titania NP provides a cleaner surface and a narrower size distribution than dispersed

particles from powders; this helps to study the transformation and transport processes (e.g. sorption and aggregation) in a more mechanistic way.

Results and discussion

Measurement of zeta potential and iso-electric point.

Titania NP are positively charged at pH below the iso-electric point (IEP). The IEP was determined using zeta potentials obtained from electrophoretic mobility measurements (EPM) using Henry's equation with correction suggested by Ohshima to transition to Schmolukowski's equation. The results obtained for the isoelectric points for two batches of titania at pH 3 and one of the "charged-reversed" batch are presented in figure 1. In Figure 1 it is possible to identify the apparent variation of IEP with different starting size; since the system is unstable close to the IEP then the measured zeta-potentials are an ensemble average of the PSD of the aggregates present. The determination of zeta potential from EPM was done using the z-average diameter and, therefore, it is not possible to distinguish the relative amount of smaller aggregates and their relative importance on the measured surface potential due to possible size-dependant surface charge⁹.

Adsorption of macromolecules on titania NP surface.

The adsorption of NOM to the surfaces of the particles was done in small volumes and low concentrations. Table 1 presents the distribution of zeta potentials and z-averaged hydrodynamic diameters at different pH and additions of the 3 different types of NOM. Figure 2 presents the characterization data as a function of HA/titania mass fraction, which shows the destabilization of the particles at low HA.

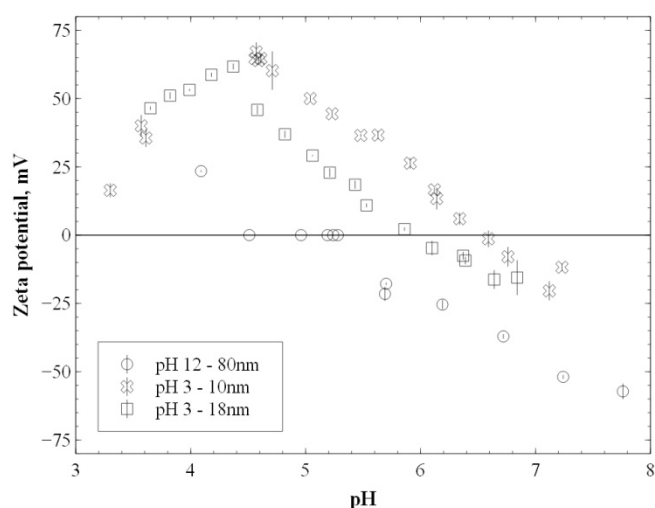


Figure 1. Zeta potential measurements as a function of pH for three different starting batches of titania NP. The suspensions were destabilized when approaching pH close to the IEP (± 2 pH units). The legend indicates starting pH and size. Error bars correspond to 1 standard deviation of three consecutive measurements once the pH had stabilized.

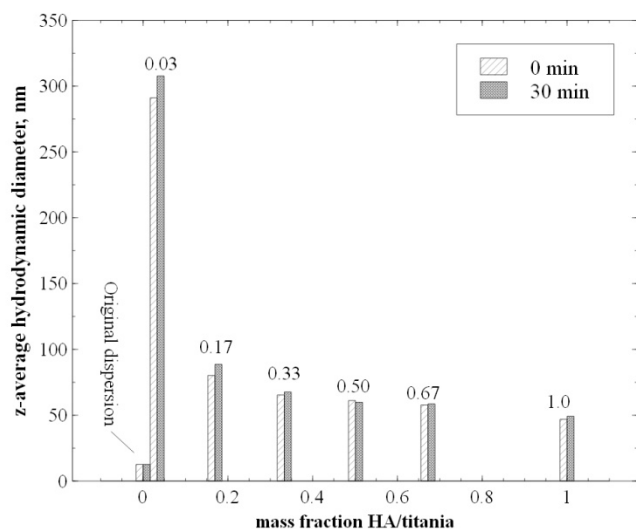


Figure 2. Change of size of titania after Humic acid addition to a varying mass ratio at pH~3.5. The labels indicate the exact mass ratio.

In figure 2, the lowest concentration of Humic acid (ratio 0.03) at low pH induced high aggregation (probably due to uncompleted surface coverage) which is also reflected in the data in table 1 for 10 mg/L HA (ratio 0.05). In table 1, 100 and 250 mg/L concentration of alginate at high pH led to zeta potentials larger than expected whilst the size remained relatively low. This could be due to that there is no attraction between the titania surface and biopolymers at this pH, and the change in zeta potential could be partly explained by signals from alginate itself. Generally, the addition of NOM in sufficient amounts to generate stable dispersions resulted in an increase of the z-averaged hydrodynamic diameter as observed in table 1 and figure 2. The charge of the particle for stabilized suspensions with HA, FA and ALG turned negative at almost all pH tested. The adsorption of macromolecules to the available sites of titania NP can occur either by surface

complexation with the carboxylic, phenolic, or hydroxyl groups on the NOM or through hydrophobic interactions. Since NOM macromolecules are polyelectrolytes with relatively low pKa (3.76 and 4.35 for FA and HA, respectively¹⁰), then the free charged groups (i.e. not complexed to the mineral surface) provides the particle with a net negative charge at the pH values tested^{11, 12}.

The evolution of hydrodynamic diameter as a function of added HA in figure 2 shows that concentrations below 16.4 mg/L HA (mass ratio 0.16) induced fast aggregation and 16.4 induced slight destabilization as it can be observed by the increase in d_H in 30 minutes. By assuming an initial d_H of ~20nm and an average MW for HA of 5.7kDa¹³ then it is possible to identify approximately the ratio of HA required to stabilize the particles. In this case 16.4 mg/L HA corresponds to 283 mol of HA per mol (part/L scaled by the Avogadro number) of particles. It is important to notice that the mixing conditions and initial concentration for the sorption of HA are very crucial: in the experiment high mixing rates were achieved and high concentrations (with respect to expected environmental particle concentrations) of particles needed to be used while in environmental context the expected concentrations can fluctuate from high (i.e. near point sources) to low (in diluted systems with diffuse emission) and shear rates can also vary. Further, the presence of natural particles will influence the behavior of the incoming particles, for instance if these enter uncoated into an aquatic compartment with elevated concentration of surfaces (particles) exposed to NOM then there is likelihood to induce heteroaggregation since there will be no time for the released NP to achieve sorption equilibrium before they get in contact with another coated particle; a similar situation was observed for polymeric coatings which increased stability between coated surfaces but enhanced deposition in uncoated surfaces¹⁴.

Table 1. Variations in zeta potential and hydrodynamic diameter measured with DLS after rapid mixing of organic molecules to 200 mg/L of titania and stabilization at still conditions for a minimum of 24 hours.

Concentration NOM, mg/L	Humic acid			Fulvic acid			Alginate		
	pH	Zeta potential mV	Size, nm	pH	Zeta potential mV	Size, nm	pH	Zeta potential mV	Size, nm
0.0	3.6	43.0	21.0	3.6	43.0	21.0	3.6	43.0	21.0
0.0	3.7	29.0	13.0	3.7	29.0	13.0	3.7	29.0	13.0
0.0	12.2	-30.0	19.0	12.2	-30.0	19.0	12.2	-30.0	19.0
10.0	3.5	16.5	261.6	3.6	31.8	789.8	4.1	35.0	133.0
100.0	3.3	-48.0	94.5	3.4	0.0	79.0	4.3	-47.0	146.0
100.0	3.6	-38.0	130.7	3.8	-28.0	112.7	4.3	-50.0	144.0
100.0	3.6	-30.0	117.3	3.8	-35.0	100.0	5.1	-70.0	138.0
100.0	4.0	-41.0	114.0	4.4	-49.0	80.0	5.5	-53.0	115.0
100.0	7.4	-32.0	50.3	7.1	-38.0	127.7	7.0	-46.0	213.0
100.0	12.2	-34.0	30.0	12.2	-40.0	28.0	12.2	-1.5	25.0
250.0	4.2	-50.0	125.0	4.7	-44.0	79.0	5.6	-78.0	250.0
250.0	12.2	-7.0	28.7	12.1	-35.0	26.0	12.3	-10.0	21.0
500.0	2.9	-42.5	96.4	3.0	-34.0	93.0	5.5	-76.0	198.0
800.0	2.8	-38.0	147.5	2.9	-37.0	190.0	5.6	-97.0	680.0

Interaction potential calculations.

The addition of different electrolytes to the dispersions of bare titania NP causes the compression of the electrical double layer that keeps the particles separated even if aleatory Brownian motion or attractive Van der Waals interactions induces collision between them. This leads to more unstable dispersions in which nanoparticles start to form aggregates at different rates depending on the level of compression of the double layer.

Calculations using the DLVO theory were made and stability ratios were determined using the approximation valid if the major contributions to the interaction potential come from the proximity of the maximum potential¹⁵:

$$W = \frac{1}{\kappa \cdot (a_i + a_j)} \cdot \exp\left(\frac{\phi_{max}}{k_B \cdot T}\right) \quad [1]$$

with k_B the Boltzmanns constant, T the absolute temperature, a_i and a_j are the radii of the particle i and j , respectively and ϕ_{max} is the height of the energy barrier. The heights of the energy barrier were calculated as the maximum in the total interaction potential $V_T(\kappa, H) = V_R(\kappa, H) + V_A(\kappa, H)$ with the subindices T , R and A for total, repulsive and attractive. The attractive potential due to Van der Waals interactions was calculated using the repulsive function for unequal spheres^{16†}. The repulsive potential due to electrostatic interactions corresponding to different pH or surface complex situations was modelled with the Derjaguin approximation for moderate potentials as a function of monovalent electrolyte concentration with the following parameters^{17†}: non-retarded Hamaker constant for TiO₂ from Gómez-Merino et al¹⁸ (anatase, 3.35E-20 J experimental), equal-sized spheres of 20 nm in diameter, temperature 293K and varying surface potential (5, 20, 40, 60 mV). The results are presented in figure 3.

Aggregation rates measurements with TR-DLS.

DLS uses the temporal evolution of the intensity fluctuations to measure the translational diffusion coefficients of particles in suspension. From the diffusion coefficient the hydrodynamic radius can be determined via the Stokes-Einstein equation. The measured hydrodynamic radius of a coagulating suspension is an average of the hydrodynamic radii of the individual aggregates, weighted by their scattered light intensities. The growth of the aggregates in a coagulation process can thus be observed by performing time-resolved DLS measurements¹⁹.

The intensity of scattered light is also dependent on the particle concentration and can be calculated as:

$$\bar{I} = \sum_k N_k \cdot I_k(q) \quad [2]$$

where N_k is the particle concentration, $I_k(q)$ is the scattered intensity of a k -fold aggregate. The result of the DLS measurement is an intensity-weighted average of the diffusion coefficient that can be expressed in the following form for the initial aggregation stages^{19, 20}:

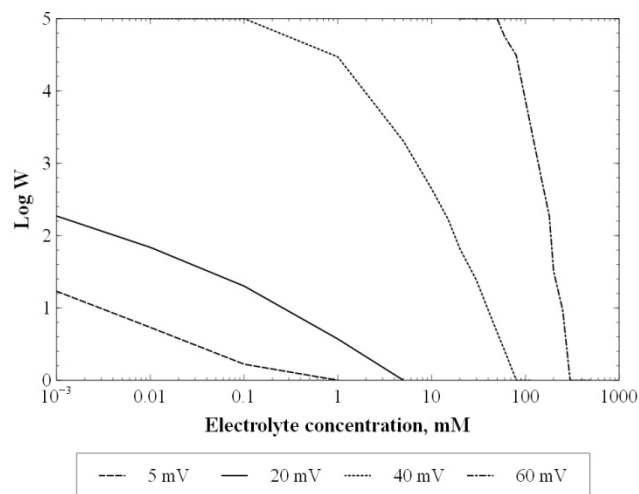


Figure 3. Theoretical stability ratios, W , between 2 spheres found with equation 5 after determining the height of the energy barrier using Derjaguin approximation. Non-retarded Hamaker coefficient 5.35E-20 J, temperature 293K, radii 10 nm. The curves are truncated in $\log(W)$ 5 and 0 to allow better visualization.

$$\bar{D} = \frac{\sum_k N_k \cdot I_k(q) \cdot D_k}{\sum_k N_k \cdot I_k(q)} \quad [3]$$

Where \bar{D} is the intensity-averaged diffusion coefficient, which is inversely proportional to the hydrodynamic radius according to Stokes-Einstein equation. The strong particle size influence on the scattering intensity will have a strong effect on the measured size, as a small amount of large particles will have such a large influence that smaller particles can be overlooked²¹. The average intensity-weighted hydrodynamic-diameter obtained by this method is called zeta-average diameter.

The combination of the above two equations and under the assumption of the limit when time tends to zero so that only duplets are formed, generates the following equation that has been widely used to determine aggregation rate constants by combining DLS and static light scattering, SLS¹⁹:

$$k_{11} = \frac{1}{N_0} \cdot \left(\left(\frac{1}{R_h(0)} \cdot \frac{d(R_h(t))}{dt} \right) \Big|_{t \rightarrow 0} \right) \cdot \left(\frac{R_{h2} - R_{h1}}{R_{h2}} \right)^{-1} - \left(\frac{1}{I_{h0}} \cdot \frac{d(I(t))}{dt} \right) \Big|_{t \rightarrow 0} \quad [4]$$

where N_0 is the initial particle number concentration, $R_h(t)$ is the hydrodynamic radius evaluated at time t , the expression $(R_{h2} - R_{h1})/R_{h2}$ corresponds to the relative change between the primary particle and the duplet.

The attachment efficiency (α), used to account for modeling the repulsive forces in solution²², can be calculated based on the aggregation rates obtained from the TR-DLS measurements, dividing the initial slope (fast aggregation) at a particular concentration with the maximum (favorable) slope, and log-plotted against salt concentration which, with enough data points available, allows to determine the critical coagulation

concentration (CCC)⁸. Another useful quantity to evaluate the stability of the suspension is the stability ratio, W . We can calculate this value with help of equation 4 and taking the maximum rate as the theoretical rate, k_{ij} , which for stable conditions or slow aggregation rates is equivalent to k_{11} but for unstable conditions can take values up to 2 orders of magnitude higher. Here it was decided to calculate k_{ij} using the difference between the first size determined with TR-DLS, R_{ha} , and the initial size, R_{hl} :

$$W = \frac{8 \cdot k_B \cdot T \cdot \left(\frac{R_{ha} + R_{hl}}{4 \cdot R_{ha} \cdot R_{hl}} \right)^2}{3 \cdot \mu} \cdot \left(\frac{1}{R_h(0)} \cdot \left. \frac{d(R_h(t))}{dt} \right|_{t \rightarrow 0} \right) \cdot \left(\frac{R_{h2} - R_{hl}}{R_{h2}} \right)^{-1} - \left(\frac{1}{I_{h0}} \cdot \left. \frac{d(I(t))}{dt} \right|_{t \rightarrow 0} \right) \quad [5]$$

Since the data here evaluated has been acquired for relatively high number concentrations, the curves obtained for the rates of increase of hydrodynamic radius (zeta-average radius) and intensities (derived count rates) have to be extrapolated to values near the time zero. The procedure followed to achieve the final results for stability ratios, W , is presented below.

Since the relative change between R_{h2} and R_{hl} is one of the parameters obtained when doing simultaneous measurements of DLS and SLS, it has to be adjusted and this is especially important for the most destabilized suspensions where the variation in intensity starts overwhelming the variation in hydrodynamic ratios. The relative change between R_{h2} and R_{hl} in equation 5 was evaluated as 1.4 (calculations have shown that the hydrodynamic radius of a duplet is approximately 1.38 times the initial diameter¹⁹) if the ratio between the initial diameter and first measurement with the calculated slope was less than 1.4. If this was not the case (as it was found for the most unstable conditions) the expression was calculated assuming R_{h2} as the radius 1 second after the mixing under the calculated slope. This allows compensating for possible deviations from the assumptions of initial duplet formation. Another compensation done in order to avoid underestimation of the stability ratios due to elevated aggregation rates was to include the total theoretical aggregation rates as explained above.

The rates of both size and intensity were plotted and the initial data points (approx. 1- 2 minutes after the start of the aggregation process) were selected for a quadratic regression with forced intercept in the initial value of diameter or intensity (this is the value before starting the aggregation process except for low rates where a slope from a linear fit was more suitable). The first derivative evaluated at time zero for these equations was then selected as the initial rate of increase and used in the equation 5 to determine the stability ratio. This was not done for cases where linearity was evident (i.e. no addition of electrolytes or some cases of low concentrations where the previous method provided misleading information).

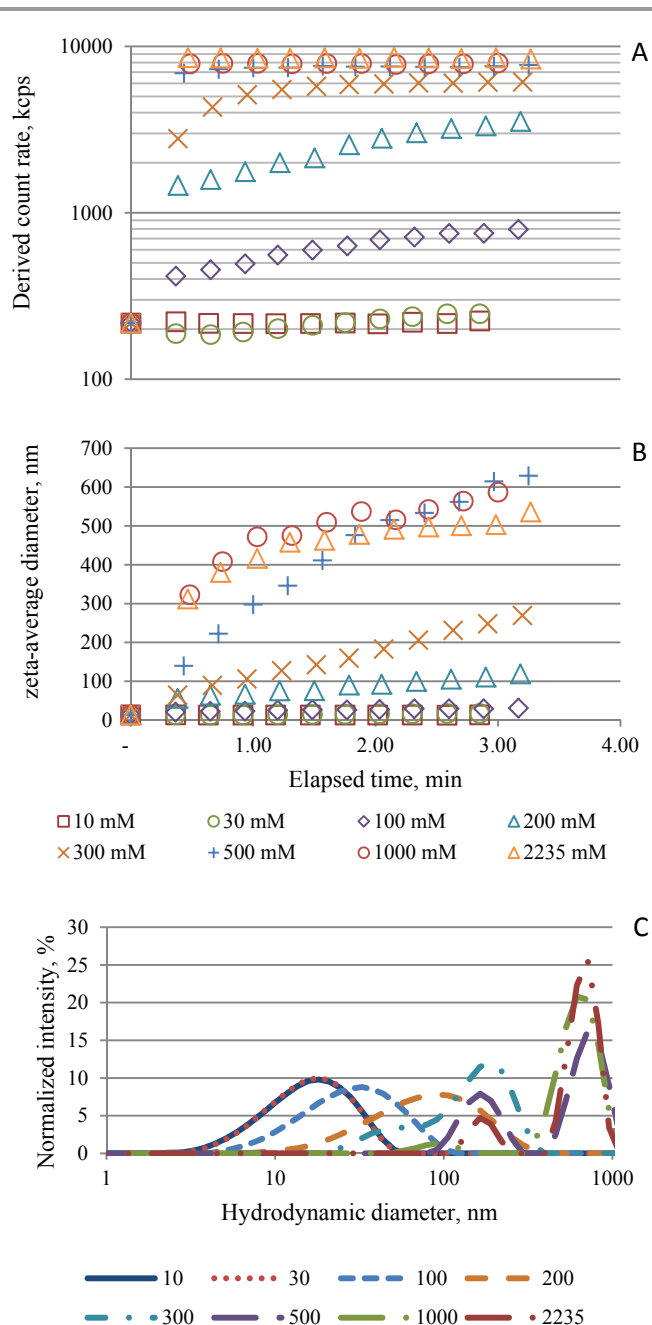


Figure 4. Measurement of aggregation rates at different concentration of NaCl. A) Mean count rates (scattered intensity) in kilo counts per second measured with DLS. B) Increase in z-average hydrodynamic diameter measured with DLS. C) Intensity-based particle size distributions using DLS measured 1 minute after the rapid mixing with different amounts of NaCl.

Figure 4 presents an example of the measurement of the rate of increase in size (Figure 4A) and scattered intensity (Figure 4B) of a dispersion of 100 mg/L titania NP at pH 3 (initial particle size 13 nm) after addition and rapid mixing of different concentrations of NaCl. Figure 4C corresponds to the PSD (based on intensity and calculated using CONTIN algorithm) measured with DLS for the same dispersions 1 minute after addition of different concentrations of NaCl. The size

distributions get broader and flatter after the addition of the electrolyte and some bimodal peaks can be distinguished for concentrations larger than 300 mM NaCl.

The stability ratios, W , were calculated as described above for different additions of the NOM and rapid mixing of the electrolytes at different concentrations. Figure 5 presents the results for 100 mg/L NOM addition (HA, FA and ALG) at different levels of NaCl, CaCl₂ and Na₂SO₄. The particle number concentrations were calculated with the hydrodynamic diameter assuming spherical particles, density of titania found in literature (3900 kg/m³) and the initial mass concentration (100 mg/L).

In the figure S1 in the SI† the stability ratios for different additions of NOM (10, 100, 250, 500 and 800 mg/L) at a limited set of pH values can be found.

In general, all additions of electrolytes without NOM followed the DLVO theory. Positively charged NP at low pH were more prone to destabilization by SO₄²⁻ compared to Cl⁻ and the opposite holds for Ca²⁺ compared to Na⁺ at high pH. If the pH of the suspension is away from the IEP the particles have a higher charge (either positive or negative) and this leads to slower aggregation rates at similar particle concentration than particles closer to IEP.

The behaviour of the experimental stability ratios in figure 5 is in accordance to DLVO theory; and even the order of magnitude in absence of NOM (figure 5, upper panels) match those calculated at different surface charge presented in figure 3.

The addition of NOM at concentrations which generated stable suspensions increased the stability of the systems with respect to NaCl and Na₂SO₄ but did not have much influence on the CaCl₂ systems. This suggests that there bridging coagulation between the organic molecules with calcium. Calcium is known to bridge the molecules in the alginate network (β -D-mannuronate and α -L-guluronate) to form gel like aggregates. Particles with HA have also shown low stability in the presence of calcium²³. Other studies with silica particles (150 nm z-average) have shown that Ca²⁺ ions destabilize the suspension in the presence of alginate and humic acid but not with fulvic acid²⁴.

The stability of FA coated NP in the presence of CaCl₂ was slightly better than for any of the other NOM sources. This is in accordance to other studies and can be due to less affinity of FA to form complex with Ca²⁺²⁵.

The weight-averaged molecular weight, MW, of SR-HA is about 5700 Da¹³, the average molecular weight of FA is 550 Da²⁶ and for Sodium alginate between 75 and 200 kDa (provided by the manufacturer). A big part of the MW of NOM is therefore due to HA and comparing the effects of this with FA in the stability of titania NP it is possible to see that small amounts of FA are more favourable for stability but as more molecules are added, HA improves the stability probably due to increased steric effects.

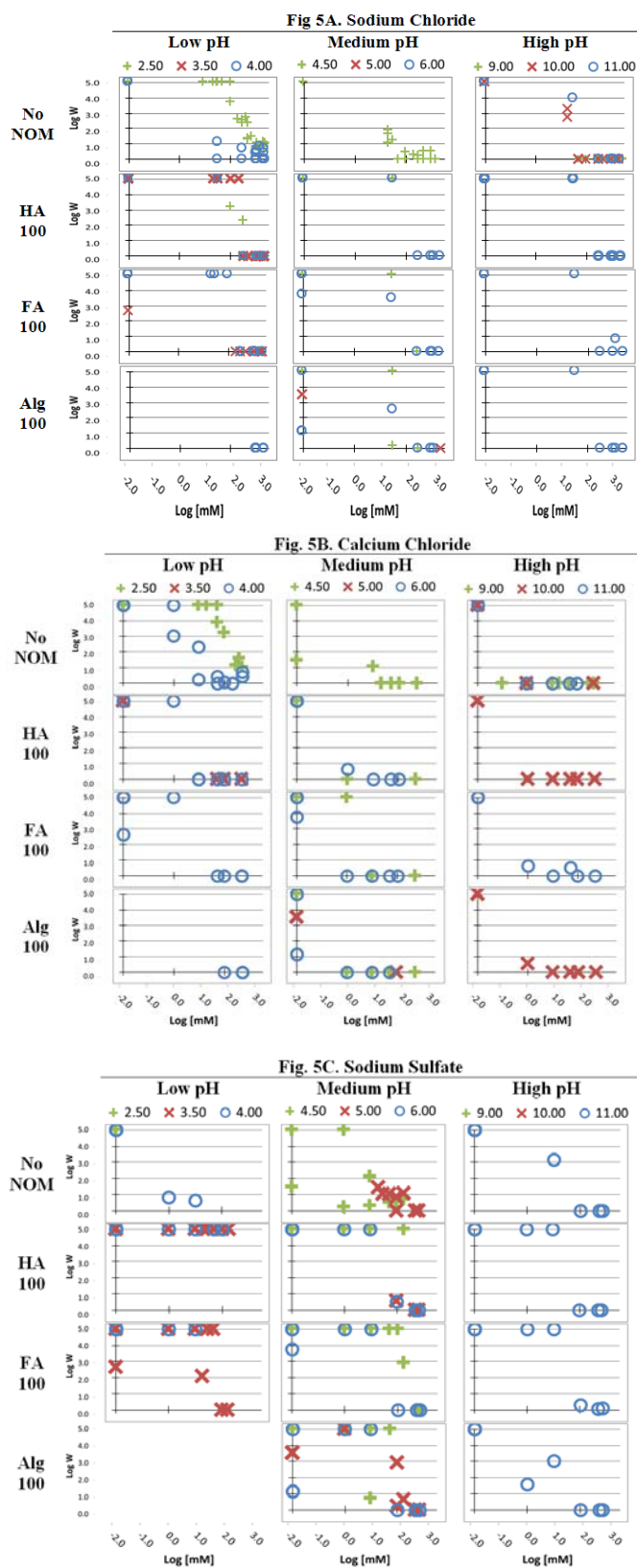


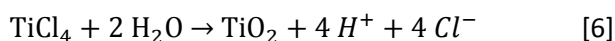
Figure 5. Stability ratio, W , at different values of pH (columns and symbols), organic macromolecules (rows) and salt concentration. A) Sodium chloride B) calcium chloride. C) sodium sulfate. The concentration of organic molecules and titania NP were kept at 100 mg/L. Equation 4 was used to determine W .

The presence of NOM increased the stability in the presence of Na_2SO_4 in a more efficient way than for the other 2 electrolytes. HA was less effective in stabilizing NP at low pH due to the fact that the carboxylic groups were protonated and, therefore, the overall surface charge were low and there was reduced electrostatic contribution to the repulsion. FA was better stabilizer even at low pH probably because there were fewer branches available for bridging coagulation with other particles and the larger overall charge density per mass of FA compared to HA (figure S2 in SI[†]).

Experimental

Synthesis of titania NP

The synthesis of TiO_2 was done via the low temperature hydrolysis of TiCl_4 , following previous publications²⁷, which proceeds according to the reaction:



Particle growth according to a specific target size was facilitated through temperature control during dialysis and solution storage, with lower temperature for smaller particles. Dialysis was done to reduce the concentration of Cl^- ions which screen TiO_2 surface charge and contribute to particle aggregation²⁷. The suspensions obtained by this method have concentrations of approximately 19 g/L of mostly anatase crystal form and the pH is around 2.5. Even though some Cl^- ions and Ti species may remain in solution after dialysis, the expected concentrations estimated are negligible after dilution to the concentrations used in this work²⁸.

In order to perform the experiments at high pH, the surface charge of the NP was modified using a fast addition of NaOH, changing the pH to around 12. One mL of the original suspension of TiO_2 was diluted with 5 mL of water and then quickly mixed with 1 mL of NaOH 0.1M. Because this process of “charge reversal” includes passing through the IEP some of the particles collide and attach to each other forming larger aggregates resulting in z-average hydrodynamic diameter of approximately 60 nm as measured by DLS.

Natural organic macromolecules

The representative NOM model compounds chosen were humic and fulvic acids (isolated from Suwanee River) and sodium alginate (isolated from brown algae). HA and FA are typical standard sources of natural organic matter used with the intention to have a material that is well characterized in order to increment the repeatability from laboratory studies; HA and FA were obtained from the International Humic Substances Society (IHSS, University of Minnesota, St. Paul, MN, USA) batches 2S101H and 2S101F, respectively. Sodium alginate (Pronova® UP LVG; chemical name Sodium (1->4)-linked β -D-mannuronate and α -L-guluronate) was obtained from Novamatrix (Sandvika, Norway). Additional information about

the physico-chemical characteristics of the NOM sources is provided in SI[†].

The particles were mixed with NOM to obtain a final mass concentration of titania of 200 mg/L and these batches were stored for a maximum of two weeks at 4C in dark before mixing in proportion 1:2 with the corresponding electrolyte prior TR-DLS measurements.

Dynamic light scattering

A DLS unit (Malvern Zetasizer, Malvern instruments Ltd, Worcestershire, UK) equipped with a 633 nm laser source was used for the measurements of electrophoretic mobility, zeta-averaged hydrodynamic diameters and the photons counts per second (count rates) of the initial conditions.

The measurements of TR-DLS were done with the Malvern Zetasizer as well. The optimum concentration that was obtained for the measurements of aggregation rates with DLS was 100 mg/L TiO_2 ; this concentration gave good statistical data at low particle diameters with the drawback of having fast aggregation rates. The measurements were done using standard operational procedures that have fixed attenuator and measurement position optimized for each set of samples. All the liquids were equilibrated at room temperature and the instrument was adjusted to that temperature as well. The refractive index and viscosity of the solutions of NaCl and CaCl_2 were obtained from the Zetasizer software and the values for Na_2SO_4 were obtained from literature^{29, 30}. The stabilized particles were thoroughly mixed during approximately 5 seconds and placed inside the instrument before the first measurement was produced; the time elapsed between initial mixing and the first measurement was noted and used as the first time point.

Influential outlier data-points in the datasets were removed if the variation in the outcome slope and intercept were several orders of magnitude larger than the ones obtained when these points were included in the calculations.

The chemicals used in this study were analytical grade acquired from Fischer Scientific.

Conclusions

The aggregation of ENP has a strong influence in the final fate of these in the environment. Here we have shown that a low coverage of NOM on top of bare titania particles can induce aggregation but further coverage can protect them from aggregating even at high ionic strengths; particles stabilized in this way are larger in size and have negative surface charge probably coming from the different functional groups in the macromolecules. The degree of coverage is going to be governed by the concentration ratio between NP and NOM and different approaches in the process of mixing can lead to different states of aggregation:

Higher initial number concentrations of particles will, generally, lead to larger sizes of the stabilized particles in the dispersion.

NOM sorption increases size, probably from small aggregate formation during the mixing process, and lowers the zeta-

potential.

Initial mixing strength (gradients) will influence the contact between particles and other additives.

The ionic composition strongly influences the aggregation behaviour, with divalent anions and cations destabilizing more strongly positively and negatively charged titania, respectively. This is in accordance to Schulze-Hardy rule that states that, depending on conditions, the CCC of a colloidal dispersion is inversely proportional to the 2nd to 6th power of the counterion valence³¹. The pH of the suspension is a major influential factor that will determine the initial charge of the NP and the degree of electrostatic repulsion. pH levels near the IEP of the ENP will lower the absolute value of the surface charge of the particle, with direct consequences on stability.

Besides the ionic strength and pH, the quality and quantity of NOM have a significant influence on the aggregation behaviour of titania. Larger molecules like humic acid and alginic acid will increase steric repulsion in the presence of NaCl and Na₂SO₄ but will destabilize strongly the particles in presence of CaCl₂ probably due to the bridging of some parts of the macromolecules with aid of Ca bridges. Therefore, in natural waters with high contents of Ca²⁺ and NOM it could be expected that the NP will undergo more aggregation than in other types of water³².

The concentrations of titania tested induced high aggregations rates. At larger stages of the aggregation process the assumption of sphericity and monodispersity is broken and the values for collision rates (k_{11}) are no longer valid. More sensitive methods for lower concentrations should be developed.

Additionally, the measurements with TR-DLS provide intensity-weighted size averages that can be significantly higher than the number-averaged values and, therefore, attachment efficiencies calculated by this method will probably lead to overestimation of aggregation rates.

Implications of this study are important mainly for near source emissions, spill situations and ecotoxicity tests, where other particles are relatively less important. In ecotoxicity tests the concentrations used differ in several orders of magnitude and, therefore, the measurement of aggregation rates using TR-DLS is more adequate compared to fate studies where the expected concentrations usually fall below the detections limits of DLS. Due to the dependence of aggregation rates on number concentrations, the actual PSD during an ecotoxicity test is variable with time and the exposure to smaller particles, which is dynamic across different treatments, will differ from the actual mass concentration. Modelling using probabilistic approaches for aggregate size distributions dynamics could be helpful in both ecotoxicity tests and environmental fate studies.

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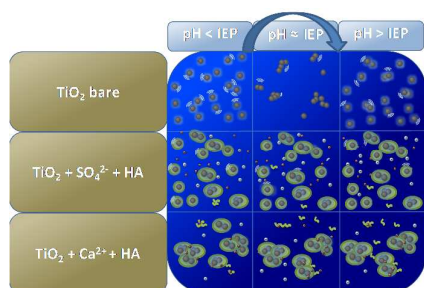
Notes and references

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† Electronic Supplementary Information (ESI) available: [Comprehensive table with other studies regarding titania stability in the presence of electrolyte or NOM; figure presenting stability ratios for other concentrations of macromolecules and equations used for the DLVO simulations; additional information about NOM sources]. See DOI: 10.1039/b000000x/

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Effects of pH, three natural macromolecules and three mono- and divalent electrolytes on the stability of titania nanoparticles